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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Rolando, BARBUCCI et al.

Serial N.. 09/830,744

Group Art Unit 1623

Filed on June 18,2001

Examiner : Krishnan , Ganapathy

For : Cross-linking Process of Carboxy-lated Polysaccharides.

DECLARATION UNDER RULE 132

I, SPORTOLETTI GIANCARLO,

DECLARE

1) *Personal data:*
Address : via Giorgio Jan n. 5 – 20129 Milan (Italy) ; tel. /fax: : +39 2 2046085.
Date and Place of Birth : 12/04/1934, Foligno (Pg), Italy
Nationality: Italian
Actual Occupation: Pharmaceutical Consultant.

2) *Education*
- Degree in Industrial Chemistry (101/110) at University of Milan, 1962;
- PHD degree ("Libera docenza") in Organic Chemistry , 1969;
- Many Courses in Organic Chemistry and Biochemistry;

3) *Professional curriculum:*

1962-1978: - Assistent Lecturer in Organic Chemistry at "Istituto di Chimica Organica", the Faculty of Sciences, University of Milan;
- Head of Research at "Institute for Vitamins and Hormones" of CNR (National Council of Research). Main Resarch on:Synthesis of chemically/enzymatically modified steroids; Biosynthesis and metabolism of steroidal plant products. Studies on the biological behaviour of modified Steroids.

1994-1994 - R&D Director of Italfarmaco Spa Research Centre (Cinisello, Milan, Italy): Fields of interest: Antithrombotic, Antinflammatory, Antianaemic products

1989-1994 - R&D Director of Ellem Srl (Farmitalia Group) Research Centre (Corsico,Milan) Fields of interest: Immunological peptide derivatives ;Wound Healing Peptides

1994-present: R&D Consultant for several Pharmaceutical Companies, *inter alia* : *Farmila Farmaceutici Milano* Spa (Settimo Milanese, Milan) and *Aquisitio* Spa , Financial Company holding interest in Pharmaceutical and Biotechnology

Fields (Milan).

4) *Scientific Production:* more than 60 papers in reviewed journals

5) *Patents :* 18 world wide patents.

6) *Affiliation:* chemical, pharmacological and scientific Societies world wide.

In this capacity , I supervised the hereinunder described experiment in order to compare the "cross-linking of carboxyalkyl celluloses (CMCs) using diamines and polyamines " method claimed by Kimberly-Clark (Quin ,Jian – EP 0566118 A1) and the cross-linking method on the same CMC, as disclosed in the Patent Application 09/830,744.

Quin (Example 1/ EP 0566118 A1), together with cross-linked products obtained by esterification of CMC (AQUALON CMC 9H4F ; sodium salt, Hercules Inc.) with di- or polyols, discloses the reaction of CMC with diethylentriamine as example of amidation cross-linking . Quin, however, (page 5, lines 57–58; page. 6, lines 1-8) hypothesizes also the formation of self-crosslinking by intermolecular esterification of the polysaccharidic carboxy and hydroxyl groups ,during the heat-treating process. The products 47 and 48 (table 1) are characterized only by *AUL Value*. No physical-chemical evidences on the kind of crosslinking bonds are reported.

In order to verify if using:
- hexandiamine as cross-linking reagent (representing the diamines used by Barbucci et al. - in US Patent Application n.830,744 - to reticulate CMC and other carboxypolysaccharides by amidation , after activation of the carboxy groups);
- the same experimental conditions (temperature, heating time,pH) described in Example 1, Tab1, and Example 2 , Tab2, of Quin's Patent (s.under) ,
the following products could be obtained:
i) a cross-linked CMC characterized by ***pure amidation***;
ii) a ***mixed*** crosslinked CMC characterized by the presence of amidic and ester bonds,
the following experiments were carried out.

EXPERIMENTAL

The following four samples were prepared:

1) AQUALON CMC 9H4F , sodium salt (Hercules Inc) 2% (w/v) solution [25g] + hexamethylendiamine 0,5% (w/v) solution [2 ml] Weigth ratio amine / CMC : 2% . pH value : 12.

2) AQUALON CMC 9H4F , sodium salt (Hercules Inc) 2% (w/v) solution [25g] + hexamethylendiamine 0,5% (w/v) solution, acified by HCl [2 ml] Weigth ratio amine/CMC : 2% . pH value : 6,5

3) AQUALON CMC 9H4F , sodium salt (Hercules Inc) 2% (w/v) solution [25g] + hexamethylendiamine 0,5% (w/v) solution [2 ml] Weigth ratio amine/CMC : 2% . The mixture was acidified by HNO_3 65% ; final pH value: 3,5

4) AQUALON CMC 9H4F , sodium salt (Hercules Inc) 2% (w/v) solution [25g] + HNO_3 65% ; final pH value: 3,5

According to Quin's Patent all solid samples were obtained by evaporative drying at 80°C in an air-convection oven (4 hrs).As Quin teaches, to performe crosslinking, 20 mg of every sample were pre-conditioned at 80°C and the temperature was quickly increased to 150°C. This temperature was mantained for 30'. The samples were analysed by FT-IR (Bruker IFS 25) and for solubility in water.

RESULTS

- The FT-IR spectra of samples 1 and 2 were very similar to that of starting CMC; (Encl. 1,2,3), moreover the two samples were ***water soluble . The data point out the absence (or the presence of a very reduced quantity) of new (crosslinking) bonds.***
- Samples 3 and 4 were ***water insoluble.***
- The FT-IR spectrum of sample 3 (Encl. 4) showed a strong band at 1747 cm^{-1} (***Ester groups***) (as reference see: Encl. 5 : FT-IR of Cellulose acetate). To verify the presence of amide band (1630-1740 cm^{-1}), avoiding the interference due to ionized carboxyl groups (1580-1730 cm^{-1}), the sample was acidified (HCl) and ***the always water insoluble*** product was submitted, after drying, to FT-IR (Encl.6). In this condition the band corresponding to ***non ionized*** carboxyl groups (1736 cm^{-1}) and that related to the ***amide groups*** (1631 cm^{-1}) appeared, while the ester band disappeared. ***This fact explains the insolubility of the sample after acidic treatment : the ester bond is hydrolised in acid milieu but not the amidic bond (as well known).***
- Sample 4 (without hexamethylendiamine) was water insoluble and the FT-IR spectrum (Encl 7) showed a band at 1724 cm^{-1} (possible overlapping of the bands corresponding to carboxyl groups partially esterified by ***sel-crosslinking***).

CONCLUSIONS

-The samples 1 and 2 obtained by the method of Quin's Patent (like hexamethylene diamine) in neutral / alkaline milieu ***does not produce amidic crosslinked derivatives*** or ***ester sel-crosslinked*** products , while in acidic milieu are obtained ***only mixed crosslinked derivatives*** (the amide bond deriving from diamine and ester bond from self-crosslinking) and ***not pure crosslinked polysaccharide products.***

This result confirms that the ***crosslinked polysaccharides*** containing ***only amidic*** crosslinking bonds ,as obtained by Barbucci's method , are *per se* a different class of derivatives in comparison with Quin's products.

I further declare that all statements of my own knowledge made herein are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that wilful false statements and the like so made are punishable by fine or imprisonment ,or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon

Milan , December 9, 2002

Giancarlo Sportoletti

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